IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of: Clendennen et al.
Application No. 10/539,488
Filed: March 13, 2006
Confirmation No. 5163
For: GENERATION OF PLANTS WITH ALTERED OIL CONTENT
Examiner: Elizabeth F. McElwain
Art Unit: 1638
Attorney Reference No. 8176-71295-07

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DECLARATION OF DR. D. RY WAGNER UNDER 37 C.F.R. § 1.132

1. I, Dr. D. Ry Wagner, hold the position of Vice President of Research, at Exelixis Plant Sciences, Portland, Oregon. I have a Ph.D. in Genetics and twenty-five (25) years of experience working in the field of plant physiology. I have performed or supervised the experiments described herein, which are an extension of the work described in the above-referenced application.

2. I have participated in experiments demonstrating that transgenic plants overexpressing the polypeptide HIO102 (SEQ ID NO:2) exhibit increased oil production without a significant increase in the proportion of long chain fatty acid (LCFA) components (*i.e.*, no significant change in oil quality).

3. To compare seed oil quality, forty-three (43) homozygous plants from the T3 generation of the *Arabidopsis* ACTTAG line IN023338 (overexpressing HIO102) and forty-two (42) wild-type control plants were grown in separate 72-cell trays. The plants were allowed to grow to maturity, self-pollinate, and set seed. Seed oil quality was determined by gas chromatography fatty acid methyl ester (GC-FAME) analysis.

4. Lipids were extracted from the seeds and trans-esterified in 500 µl 2.5% H_2SO_4 in MeOH for 3 hours at 80 °C, following the method of Browse *et al.* (*Biochem. J.* 235:25-31, 1986), with modifications. A known amount of heptadecanoic acid was included in the reaction as an internal standard. 750 µl of water and 400 µl of hexane were added to each vial, which was then shaken vigorously and allowed to phase separate. Reaction vials were loaded directly onto GC for analysis and the upper hexane phase was sampled by the autosampler. Gas chromatography with Flame Ionization detection was used to separate and quantify the fatty acid methyl esters. Agilent 6890 Plus GC's were used for separation with Agilent Innowax columns (30 m x 0.25 mm ID, 250 µm film thickness). The carrier gas was hydrogen at a constant flow of 2.5 ml/minute. 1µl of sample was injected in splitless mode (inlet temperature 220 °C, purge flow 15 ml/min at 1 minute). The oven was programmed for an initial temperature of 105 °C, initial time 0.5 minutes, followed by a ramp of 60 °C/minute to 175 °C, and a 40 °C/minute ramp to 260 °C, with a final hold time of 2 minutes. Detection was by Flame Ionization (temperature 275 °C, fuel flow 30.0 ml/min, oxidizer 400.0 ml/min). Instrument control and data collection and analysis were monitored using the Millennium Chromatography Management System (Version 3.2, Waters Corporation, Milford, MA). Integration and quantification were performed automatically, but all analyses were subsequently examined manually to verify correct peak identification and acceptable signal to noise ratio before inclusion of the derived results in the study.

5. The relative amount of individual fatty acids in each sample was determined by dividing the amount of a fatty acid by the sum of the total fatty acids identified by GC-FAME analysis. The proportion of each of 16:0, 18:0, 18:1, 18:2, 18:3, 20:0, 20:1, 22:0, and 22:1 LCFAs expressed as a percentage of the total fatty acid content in control plants and transgenic plants with altered expression of HIO102 are plotted on the histogram submitted herewith as **Exhibit A**. To compare the amount of individual fatty acids in the seed from HIO102 and control plants, the average and standard deviation were calculated for the relative amount of each fatty acid from the seed of the 43 HIO102 plants and 42 control plants. No significant difference in the proportion of individual fatty acids was observed as indicated by similar average values and overlapping standard error bars for each component.

6. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under 18 U.S.C. § 1001, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

D. Ry Wagner, Ph.D.

12-18-08
Date